

LOI:

1) Proposal Title: Evolution of Autotrophy

2) List of participants:

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3) Brief Description of Proposed Program:

Earth was formed 4.5 Ga bp. Evidence for biological activities is dated to between 3.8 to 3.5 Ga bp. This first life was microbial and most likely evolved in deep ocean sediments or in subterranean environments because those environments were protected from sterilization by bolide impacts during the period of heavy bombardment which occurred during the 500 million years after Earth formed.

Although the nature of the first types of metabolism are unknown, the evolution of life likely occurred in subterranean environments such as found in the Homestake Mine because they were more protected from bombardments than surface environments.

Autotrophy (whereby carbon dioxide gas is fixed into organic carbon) was a major evolutionary breakthrough which likely occurred about 3.5 Ga bp. Autotrophy was important on Early Earth because it provided a biological mechanism for synthesis of organic material. Thus, on Earth today, photosynthesis or photoautotrophy provides the vast majority of organic matter that is used for living organisms. However, photoautotrophy is not thought to have been the first type of autotrophy on early Earth because environments that were exposed to light were susceptible to asteroid impacts. For this reason, most scientists believe that chemoautotrophy or chemosynthesis was the first type of autotrophic metabolism.

Chemoautotrophy relies on the oxidation of reduced inorganic chemicals for an energy source. Hydrogen gas, sulfide and other reduced materials are known to serve as energy sources for chemosynthesis and are materials that are found in subterranean environments.

On the present-day Earth, chemoautotrophy occurs in total darkness at some hydrothermal vent areas on the ocean bottom, where gases from the Earth's interior escape into the water at elevated temperatures. A similar situation may have occurred billions of years ago in what is now the Homestake Mine, since there is fossil evidence of ancient hydrothermal vents in the mine. It is likely that chemoautotrophic microorganisms grew and evolved near these ancient vents. Chemoautotrophic descendants of these original vent microorganisms may still exist in some areas of Homestake. Once the nature of the chemoautotrophs in Homestake is understood, comparisons can be made between them and the chemoautotrophs near modern deep sea hydrothermal vents, to see what similarities and differences there are. Comparisons

would also be made between Homestake's chemoautotrophs and those that have been found in other terrestrial deep subsurface environments on the modern-day Earth.

The chemoautotrophic bacteria in Homestake are important in that they likely provide the base of the microbial ecosystem in the mine, being the main primary producers. In order to fully understand the microbial processes in the mine, it is important that the nature of the mine's chemoautotrophs be understood.

Hypotheses of this study are that:

- 1) The types of chemoautotrophy that occur in Homestake Mine have evolved from the earliest autotrophic metabolisms that occurred on Earth. Their genome sequences will suggest this. By studying these microorganisms, we may learn much about the evolution of this important process.
- 2) Chemoautotrophic bacteria are the main primary producers of the microbial ecosystem in Homestake.
- 3) The genomes of the chemoautotrophic bacteria in Homestake will have some sequences and/or features in common with both terrestrial and marine chemoautotrophs on the present-day Earth.

Science goals of project:

1. Enrich and attempt to isolate pure cultures of chemoautotrophic microorganisms from samples taken throughout the mine. In particular, anaerobic autotrophic microorganisms will be grown for investigation. These would include methanogens and sulfur- and sulfate-reducing bacteria. However, we expect that enrichments will also yield novel metabolisms and organisms.
2. Assay for autotrophic metabolisms and identify autotrophs using stable and radiolabeled carbon dioxide.
3. Collaborate with Terry Hazen and others on the eco-genomic studies. One approach would be to identify genes for key enzymes known to be involved in chemoautotrophy such as Rubisco, etc.. The other approach would be to carry out eco-genomics using biomass from the mine that been labeled with ^{13}C - carbon dioxide, the assumption being that the autotrophs will uptake this earlier than the heterotrophs which will rely on the organics produced by the autotrophs. The heavy labeled DNA will be used for eco-genomics.

4. Infrastructure requirements

1. Laboratory facilities at the mine for collection and treatment of samples are as proposed by the Geomicrobiology group that has submitted other LOIs, including a laboratory facility at BSL2 level in the mine to allow some processing of samples, and to allow some of the microbial work to be done soon after sampling. This lab facility would be available to other workers/researchers in the mine for their needs.
2. Some sample processing would take place in laboratories at university and other research sites at a distance from the mine.

Both J. T. Staley and B. H. Bleakley would each have need for a post doctoral researcher, technical help, and supplies (O & M) to carry out the work at their home institutions, and when sampling and analyzing samples from Homestake.

5. Readiness for deployment: technology

We would be able to sample mine waters at the time of re-entry before official opening. Access sometime in 2006 would be desirable.

6. Readiness for deployment: effort and funding:

Efforts would begin in 2006 to write and submit grants to fund the proposed work. Some preliminary sampling and characterization of microbial isolates and communities could be done without major additions of new funds and resources. But new personnel (graduate students, etc.) and O & M funds to help them do their work will be needed to move this work forward.

7. Environment, safety and health issues/hazards

No specific problems are anticipated.